Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
Department of Analytical Chemistry

Candidate: Lucie Pincová

Supervisor: Doc. RNDr. Marie Pospíšilová, CSc.

Title of Diploma Thesis: Analysis of aesculin, aesculetin and phenylephrine in eye drops by micellar electrokinetic chromatography

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A new method of micellar electrokinetic chromatography with UV detection for the simultaneous determination of aesculin, aesculetin and phenylephrine was devised and validated. The separation was optimized by examining a number of experimental conditions, such as type, concentration and pH of the background electrolyte, the addition of a surface-active agent (sodium dodecyl sulfate, SDS) and methanol and effect of applied voltage and operating temperature. The separation was carried out in a fused-silica capillary (internal diameter 50 μm, total length 64.5 cm and effective length 8.5 cm) with UV detection at 210 nm, using hydrodynamic injection under the pressure of −50 mbar for 6 seconds. Optimal conditions for the separation and determination of the drugs were: background electrolyte 20mM boric acid of pH 8.6 (adjusted with 100mM NaOH) containing 60 mM SDS and 5 % (*V/V*) of methanol. Benzoic acid (0.1 mg/ml) was used as internal standard. The capillary temperature was maintained at 25 °C, the applied voltage was −25 kV. Calibration curves were linear in the range 10−500 μg/ml of both aesculin and aesculetin and from 12.5 to 625 μg/ml of phenylephrine; the correlation coefficients were in the range 0.9975−0.9996. The *RSD* values were in the range 0.58−1.20 % ($n = 6$) when determining 0.2 mg/ml of both aesculin and aesculetin and 0.25 mg/ml of phenylephrine in pure standard solution. The detection limits of aesculin, aesculetin and phenylephrine were 1.99; 1.32 and 3.36 μg/ml. The method was successfully applied for the assay of aesculin and phenylephrine in a real pharmaceutical preparation (*RSD* 1.86 −1.98 %; $n = 3$). The separation took less than 3 min.